Medicinal and Aromatic Plant Science and Biotechnology ©2011 Global Science Books

Active Compounds from Ginger as Inducers of Mitochondrial Apoptotic Pathway: An *in Silico* Prediction

Ashutosh Mukherjee

Department of Botany, Dinabandhu Mahavidyalaya, Bongaon, North 24 Parganas – 743235, West Bengal, India

Corresponding author: * **ashutoshcaluniv@gmail.com**

ABSTRACT

Knowledge about the mitochondrial apoptotic pathway is very important for chemotherapeutic drug development. The enzyme NADH dehydrogenase can be an important target for natural drugs in this respect. Active compounds of ginger (*Zingiber officinale* Roscoe) namely gingerol, paradol, shogaol and zingerone were selected as ginger is known to have anti-cancer properties. A three-dimensional model of the human NADH dehydrogenase was developed *in silico* and docked with gingerol, paradol, shogaol and zingerone. All of them were successfully docked. Among them, two compounds, namely 6-gingerol and 10-gingerol, formed covalent bonds with the enzyme showing that they are the most potent for drug development among the active components of ginger. The successful docking of these natural compounds with the enzyme showed that these compounds are potential inducers of the apoptotic pathway and thus important in chemotherapeutic drug development.

Keywords: chemotherapeutic drug, vanilloid, protein modeling, ligand, docking

INTRODUCTION

With the advent of new technologies, the molecular biology of cancer development as well as apoptotic pathways are becoming clear to us. Mitochondria play an important role in the internal 'intrinsic' apoptotic pathway (Dixon *et al*. 2007). Naturally, there has been a growing interest in targeting the proteins involved in mitochondrial apoptotic pathway to develop new anticancer drugs. Several plant-based drugs like capsaicin and resiniferatoxin (commonly called as vanilloids) have been shown to induce the direct apoptotic pathway in mitochondria by inhibiting the run of the electrons through the electron transport chain (Ziglioli *et al*. 2009). They inhibit the flow of electrons to coenzyme Q from NADH oxidoreductase (Complex I) which leads to determining an excess of reactive oxygen species (ROS). These ROS are responsible for DNA damage which ultimately leads to apoptosis. Thus, these plant products can be considered as coenzyme antagonists (Ziglioli *et al*. 2009).

Ginger (*Zingiber officinale* Roscoe), one of the most widely used species of the family Zingiberaceae is a common condiment for various foods and beverages due to its pungency and also has a long history of medicinal use dating back 2500 years (Shukla and Singh 2007). The pungency of ginger primarily comes from a number of vanilloid compounds viz. gingerol, shogaol, paradol etc. The fresh rhizome of ginger contains [6], [8] and [10]-gingerol (McGee 2004). Upon drying, the [6]-gingerol is converted to [6]-shogaol, which is more pungent (McGee 2004). Medicinal uses of ginger appear in early Sanskrit and Chinese medical literature as well as Roman, ancient Greek and Arabic medical texts (Mills and Bone 2000). Recently, ginger has been shown to possess anticancer properties (Hanim *et al*. 2008). The anticancer properties of ginger are attributed to the presence of these pungent vallinoids, viz. [6]-gingerol and [6]-paradol, as well as some other constituents like shogaols, zingerone etc. (Shukla and Singh 2007).

The underlying mechanism by which ginger act as an anticancer agent, is not clearly known to date. As gingerol, paradol, shogaol and zingerone possesses much structural similarity to capsaicin, they may also act by inhibiting NADH oxidoreductase (NADH dehydrogenase), thus inhibiting the electron flow. If the vanilloids from ginger bind as ligands with the enzyme, then it can be said that they can inhibit its function. The *in silico* protein-ligand interaction plays a considerable role in structure based drug designing (Daisy *et al*. 2009). Structure based drug designing has made tremendous contributions in the field of drug discovery. *In silico* molecular docking is one of the most powerful and useful techniques to discover novel ligands for receptors of known structure and thus play a major role in structure-based drug designing (Brooijmans *et al.* 2003). Establishement of ayurvedic remediation for Ebola hemorrhagic fever (Bagchi *et al.* 2009) by homology modeling with *in silico* docking and medication for Alzheimer's disease (Gore *et al*. 2010) were previously reported. Thus, *in silico* prediction of drugs can be a starting point for successful drug designing for several diseases including cancer. In this paper, an *in silico* molecular docking was done to investigate whether the active compounds from ginger could bind to NADH oxidoreductase enzyme as ligands.

MATERIALS AND METHODS

Protein selection and modeling

The Human NADH dehydrogenase [ubiquinone] flavoprotein 2 (accession no. NP_066552.2) was obtained from NCBI Refseq (Pruitt *et al*. 2007) database (http://www.ncbi.nlm.nih.gov/projects/RefSeq/). This 24 kDa subunit of complex I (The NADH-ubiquinone oxidoreductase complex) is involved in electron transfer, as described in the Refseq deatabase. This protein served as the starting material of this study. For prediction of three dimensional structure of this protein, homology modeling was done using Swiss-model (Arnold *et al*. 2006) server (http://swissmodel.expasy.org/) and EsyPred3D (Lambert *et al*. 2002) server (http://www.fundp.ac.be/sciences/biologie/urbm/bioinfo/esypred/). For the 3D modeling, appropriate templates were searched by Swiss model and submitted for modeling both in Swiss-model and EsyPred3D. In Swiss-model, protein was

Received: 16 May, 2011. Accepted: 26 July, 2011.

Original Research Paper

modeled in alignment mode; the alignment was done with Swiss PDB viewer. In EsyPred3D, the model was generated using default parameters. After modeling, the models were analyzed by RAMPAGE Ramachandran plot (Lovell *et al*. 2003) server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

Ligand preparation and docking

Chemical structures of [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shogaol, [6]-paradol and zingerone were downloaded from KEGG (Kanehisa and Goto 2000) chemical database (http://www.genome.jp/kegg/) and 3D rendering were done by ACD/ChemSketch (Freeware), release 12. The 3D rendered structures were saved as *.mol file and converted to *.pdb file using ArgusLab 4.0.1 (Thompson 2004) downloaded from http://www.arguslab.com. These structures were used as ligands for docking with the 3D modeled NADH dehydrogenase. Docking was done using PatchDock (Duhovny *et al*. 2002; Schneidman-Duhovny *et al*. 2005) server (http://bioinfo3d.cs.tau.ac.il/PatchDock/). For docking, 1.5Å clustering RMSD was chosen and Protein-small ligand was chosen as complex type. 3D structures of the protein-ligand complex were visualized with Rasmol (Sayle and Milner-White 1995).

RESULTS

Modeling of NADH dehydrogenase

The Swiss model selected the known structures of proteins 3IAMB (hydrophilic domain of respiratory complex I from *Thermus thermophilus*), 2AUVA (A thioredoxin-like ferredoxin involved in the NADP-reducing hydrogenase complex from *Desulfovibrio fructosovorans*) and 1F37B (thioredoxin-like ferredoxin from *Aquifex aeolicus*) as template. Alignment of these three and the protein of interest were aligned and modeled with Swiss model. In EsyPred3D, 3IAMB was given as the template as it showed maximum similarity with the concerned protein sequence. RAMPAGE Ramachandran plot server gave the following results of the three dimensional structure of these two modeled proteins.

The Swiss-model generated model

Number of residues in favoured region (~98.0% expected): 127 (84.1%). Number of residues in allowed region (~2.0% expected): 14 (9.3%). Number of residues in outlier region: 10 (6.6%).

The EsyPred3D generated model

Number of residues in favoured region (~98.0% expected): 141 (90.4%); Number of residues in allowed region (~2.0% expected): 9 (5.8%); Number of residues in outlier region: 6 (3.8%).

Based on these results, the second model was chosen for further analysis as it showed more residues in favoured region. The Ramachandran plot of the second model is given in **Fig. 1** and the 3D model is given in **Fig. 2**. The structure showed 5 beta sheets and 6 alpha helices.

Docking

The model obtained with EsyPred3D was subjected to docking. The active compounds from ginger were selected as ligands. All of these ligands (**Fig. 3**) have three regions: a vanillyl moiety, a region linking ester and an aliphatic region. These ligands and the protein were submitted to patchdock server. The results with highest docking score were selected and given in **Table 1**. Proteins docked with different ligands are shown in **Fig. 4**. The highest docking score was obtained with 10-gingerol (4854) and the lowest score was obtained with zingerone (3482). [10]-gingerol also acquired highest interface area of the complex (661.90). It showed an ACE (the desolvation free energies required to transfer atoms from water to a protein's interior) of -196.23,

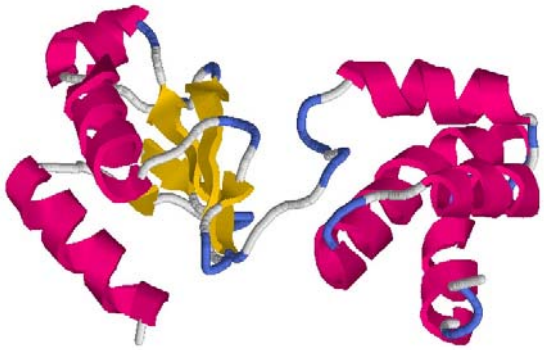

Fig. 1 Three dimensional structure of the enzyme Human NADH dehydrogenase modeled by EsyPred3D and visualized in Rasmol. The alpha helices are shown in red, beta sheets are shown in yellow, blue regions are loops and grey regions are unfolded.

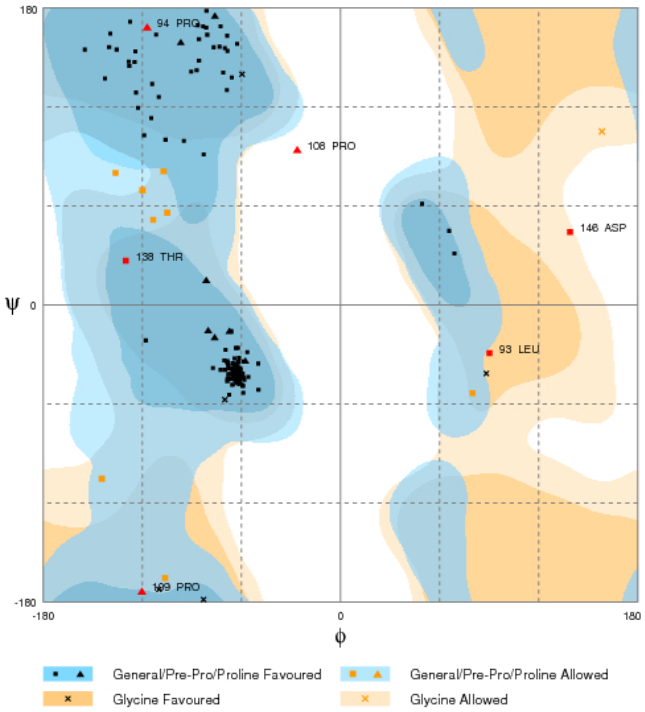

Fig. 2 Ramachandran plot of the model of NADH dehydrogenase obtained by Rampage.

Table 1 Results of docking as given by the Patchdock server.

Name of the ligand	Score[1]	Area[2]	ACE[3]
[6[-gingerol	4800	520.40	-59.29
[8]-gingerol	4728	555.70	-123.74
[10]-gingerol	4854	661.90	-196.23
[6]-paradol	4602	548.50	-115.86
[6]-shogaol	4602	567.00	-133.28
Zingerone	3482	415.60	-93.30

[1]Geometric shape complementarity score (Duhovny *et al*. 2002)
[2]Approximate interface area of the complex
[3]Atomic contact energy (according to Zhang *et al*. 1997)

the lowest among all. The lowest interface area was shown by zingerone (415.60). Among all the compounds, only [6] and [10]-gingerol showed covalent bonding with the NADH dehydrogenase enzyme (**Fig. 5**). [6]-gingerol was bound with lysine 167 of the chain (part of a loop between two beta sheets) with the terminal region of its aliphatic chain. The terminal three carbon atoms and the associated hydrogen atoms of the aliphatic chain of [6]-gingerol was bound with the nitrogen and two carbon atoms of the side chain of lysine. [10]-gingerol was bound with Asparagine 90, Asparagine 191 and tyrosine 192. Asparagine 90 was the part

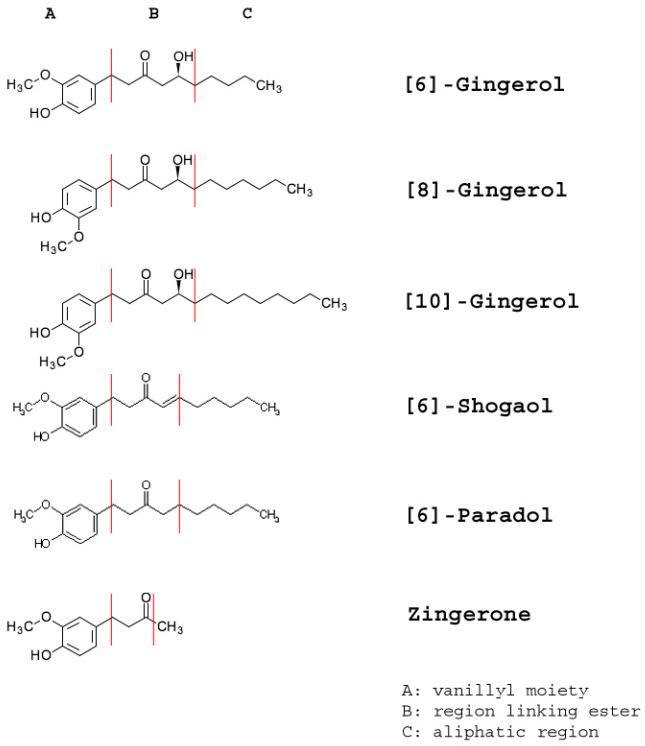

Fig. 3 Chemical structures of the ligands used in this study. All of them have three functional regions: one vanillyl moiety, a region linking ester and an aliphatic region. In zingerone, the aliphatic region is represented only by a methyl group.

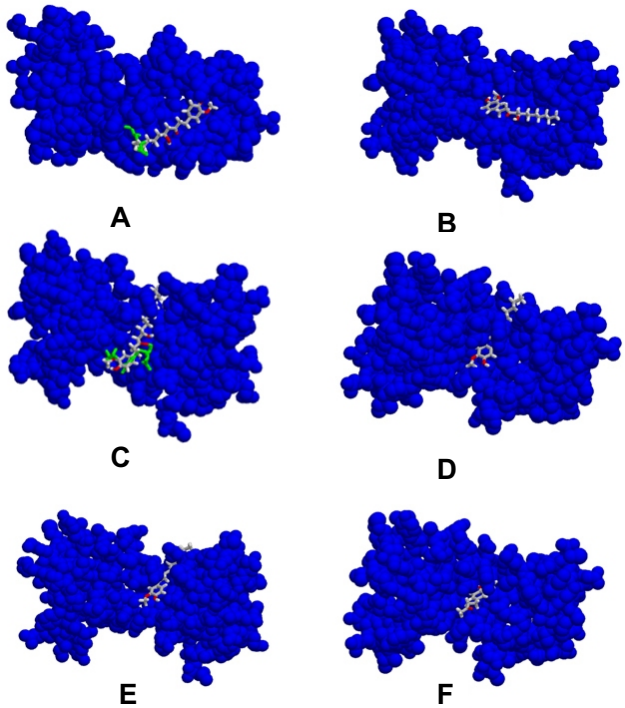

Fig. 4 Protein-ligand complexes docked by PatchDock. the protein is represented in space-fill model in blue. The ligands are represented in sticks. The ligands are the following: (**A**) [6]-gingerol; (**B**) [8]-gingerol; (**C**) [10]-gingerol; (**D**) [6]-shogaol; (**E**) [6]-paradol and (**F**) zingerone. Green portions in (**A**) and (**C**) indicate amino acids bound with the ligands.

of one alpha helix and the other two amino acids were the part of a loop. The aromatic ring of the vanillyl moiety was involved in bonding with Asparagine 90 and Tyrosine 192. Asparagine 191 was bonded with the aliphatic chain of the ligand.

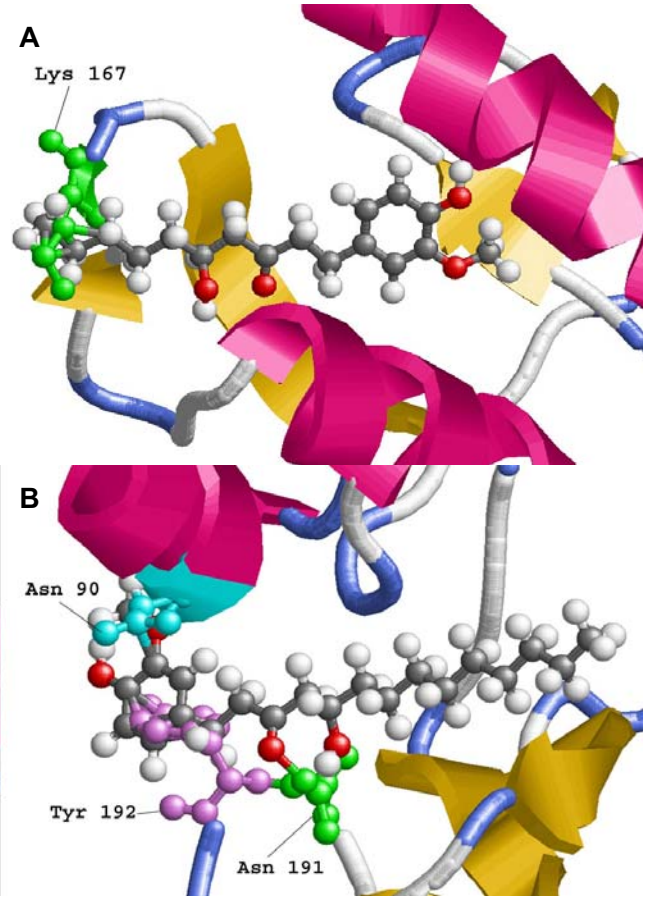

Fig. 5 Ligand binding pocket of the enzyme bound with ligands. (**A**) with [6]-paradol, Lysine 167 is represented in green; (**B**) with [10]-gingerol, Asparagine 90, Asparagine 191 and Tyrosine 192 are represented in cyan, green and violet, respectively. In both cases, ligands are represented in ball and stick model.

DISCUSSION

Natural products are considered as a rich source of compounds that embrace many applications in the fields of pharmacy (Gordaliza 2007; Coseri 2009). Eukaryotic cells are complex and offer compartmentalised macromolecular targets for interaction with natural products (Dixon *et al*. 2007). The interaction of natural products with certain specific eukaryotic cellular targets has been widely studied which are important in the context of pharmaceutical development (Dixon *et al*. 2007; Dairaku *et al.* 2010; Xia *et al.* 2011). Mitochondria play an important role in the intrinsic apoptotic pathway (Papa and Skulachev 1997; Joza *et al.* 2001; Wang and Youle 2009). Relationship between dysfunctional apoptosis and oncogenesis has made mitochondria as a target of intense research (Schroeter *et al* 2003; Dhanasekaran and Reddy 2008; Kang *et al.* 2009; Leav *et al.* 2010; Kang *et al.* 2011). The protein-ligand interaction plays a significant role in structure-based drug designing (Andricopulo *et al.* 2009; Daisy *et al*. 2009; Waszkowycz *et al.* 2011). In the present work, the enzyme NADH dehydrogenase was taken to examine that whether it can act as a target for the active components of ginger. The active components of ginger includes [6], [8] and [10]-gingerol, [6]-shogaol, [6]-paradol and zingerone. They were also chosen to explore the difference in binding mechanism of these to the NADH dehydrogenase enzyme. The human NADH dehydrogenase [ubiquinone] flavoprotein 2 (accession no. NP_066552.2) was selected as it was obtained from NCBI Refseq database. This database contains only annotated and curated collection of nucleotide sequences (Pruitt *et al*. 2007). As no three dimensional model for NADH dehydrogenase was found, 3D modeling was employed by homology modeling with known structures. For modeling, both

Swiss model as well as EsyPred3D servers was selected as they are widely used (Iyer *et al*. 2007; Gu *et al.* 2009; Sharma and Nigam 2010; Sharma *et al.* 2011). However, the model generated by EsyPred3D was chosen for further analysis as it showed more residues in favoured region than Swiss model generated structure, as shown by the Ramachandran plot. The docking was performed with PatchDock (Schneidman-Duhovny *et al.* 2005) as no previous information about the docking pocket is required in this algorithm. It employs a technique in which the surfaces are divided into patches according to the surface shape and these patches correspond to patterns that visually differentiate between puzzle pieces (Schneidman-Duhovny *et al.* 2003). Once the patches are recognized, they can be superimposed using shape matching algorithms which notify that a hybrid of the Geometric Hashing and Pose-Clustering matching techniques are applied to match the patches detected. Concave patches are matched with convex and flat patches with any type of patches. All complexes with unacceptable penetrations of the atoms of the receptor to the atoms of the ligand are discarded. Finally, the residual candidates are ranked according to a geometric shape complementarity score (Maheswari 2011). Patchdock successfully predicted protein interactions for many examples (Gidalevitz *et al*. 2004; Inbar *et al*. 2005; Benyamini *et al*. 2006; Maheswari 2011; Sharma *et al*. 2011). Molecular docking showed that the different ligands docked with different region of the protein (**Fig. 4**) indicating that minor differences in ligand structure leads to totally different protein-ligand interaction. Docking also showed that only [6]-gingerol and [10]-gingerol got covalently attached with the enzyme. It indicates that these two compounds are the most potent components of ginger as ligands. [8]-gingerol, although similar in structure with [6] and [10]-gingerol, did not formed any bond with the amino acids of the enzyme. Even [6]-gingerol and [10]-gingerol bonded with different amino acids of the enzyme. While [6]-gingerol made bond with only one amino acid (lysine 167), [10]-gingerol bonded with three different amino acids (asparagine 90, asparagine 191 and tyrosine 192). [10]-gingerol had the longest aliphatic chain and, thus could access three amino acids for bonding and thus, showed the highest docking score (4854). The second highest docking score was shown by [6]-gingerol (4800). However, area covered by different ligands increased with the increasing size of the ligand. For example, [6], [8] and [10]-gingerol showed increasing size of the aliphatic chain. The area covered by them with the enzyme was also in the increasing order (520.40, 555.70 and 661.90 respectively) as shown in **Table 1**. Difference in docking scores with similar ligands was reported previously (Ashokan 2010). However, the docking score did not corroborate with the size. [8]-gingerol showed the much lower docking score (4728) than [6] and [10]-gingerol. This shows that covalent bonding with the protein by a ligand increase its docking score. Among others, zingerone showed lowest docking score (3482) and thus, not much suitable as a ligand for NADH dehydrogenase. Structure of [6]-shogaol and [6]-paradol varied by only a double bond in the middle region of the aliphatic chain (**Fig. 3**) and both showed the same docking score (4602) indicating that this variation has a little effect on docking. They also had similar interface area of the complex (567 for [6]-shogaol and 548.50 for [6]-paradol). The atomic contact energy (ACE) of the protein-ligand complex varied considerably with [10] and [6]-gingerol (-196.23 kcal/mol and -59.29 kcal/mol, respectively), although both had good docking scores. This may be due to the fact that [6]-gingerol occupied mostly the surface of the protein whereas [10]-gingerol was more embedded into it (**Fig. 4**). This made [10]-gingerol to interact with more amino acid side chains than [6]-gingerol which caused the difference in ACE. Thus, [10]-gingerol shows more affinity to the protein than [6]-gingerol.

The present study originated from two angles. First, ginger is known to have anti cancer properties and secondly, the active compounds have structural similarity with capsaicin, another vanilloid which has been shown to inhibit the flow of electrons to coenzyme Q from Complex I, which possess the enzyme NADH dehydrogenase (Ziglioli *et al*. 2009). The active components of ginger studied in this paper are also vanilloids and previously shown to bind with vanilloid receptors (Dedov *et al*. 2002), much like capsaicin does. These facts raised the question whether these compounds could bind to NADH dehydrogenase. The present study showed that at least two of them ([6]-gingerol and [10]-gingerol) can bind with NADH dehydrogenase. This showed the successful application of molecular docking in finding new targets for known compounds.

Although many known and newly discovered natural products are generally screened routinely for use as chemotherapeutic agents, in several instances, the molecular targets of these products are not clearly known (Rochfort 2005; Böttcher *et al.* 2010). The *in-silico* approach can be a starting point for indicating the proper molecular target of natural products. This approach helps scientists by giving them a preliminary idea so that they can advance towards the drug development (Waszkowycz *et al.* 2011). *In-silico* ligand docking is thus an important subject of research since it saves time, enables effective utilization of funds and gives well predicted results for effective utilization. Since the above study is an *in-silico* work, the compounds (especially [6]-gingerol and [10]-gingerol) have to go to clinical trials to establish their efficacy. If these compounds will succeed in clinical trials, some efficient anticancer drugs can be found.

REFERENCES

Andricopulo AD, Salum LB, Abraham DJ (2009) Structure-based drug design strategies in medicinal chemistry. *Current Topics in Medicinal Chemistry* **9 (9)**, 771-790

Arnold K, Bordoli L, Kopp J, Schwede T (2006) The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. *Bioinformatics* **22**, 195-201

Ashokan KV (2010) Docking studies on abscisic acid receptor pyrabactin receptor 1 (pyr1) and pyrabactin like receptor1 (pyl1). *International Journal of Environmental Sciences* **1 (3)**, 314-322

Bagchi P, Mahesh M, Somashekhar R (2009) Pharmaco-Informatics: Homology modelling of the target protein (GP1, 2) for Ebola hemorrhagic fever and predicting an Ayurvedic remediation of the disease. *Journal of Proteomics and Bioinformatics* **2**, 287-294

Benyamini H, Shulman-Peleg A, Wolfson HJ, Belgorodsky B, Fadeev L, Gozin M (2006) Interaction of C60-Fullerene and carboxyfullerene with proteins: Docking and binding site alignment. *Bioconjugate Chemistry* **17**, 378-386

Brooijmans N, Kuntz ID (2003) Molecular recognition and docking algorithms. *Annual Review of Biophysics and Biomolecular Structure* **32**, 335-373

Böttcher T, Pitscheider M, Sieber SA (2010) Natural products and their biological targets: proteomic and metabolomic labeling strategies. *Angewandte Chemie International Edition* **49 (15)**, 2680-2698

Coseri S (2009) Natural products and their analogues as efficient anticancer drugs. *Mini-Reviews in Medicinal Chemistry* **9 (5)**, 560-567

Dairaku I, Han Y, Yanaka N, Kato N (2010) Inhibitory effect of curcumin on IMP dehydrogenase, the target for anticancer and antiviral chemotherapy agents. *Bioscience, Biotechnology, and Biochemistry* **74**, 185-187

Daisy P, Sasikala R, Ambika A (2009) Analysis of binding properties of phosphoinositide 3-kinase through *in silico* molecular docking. *Journal of Proteomics and Bioinformatics* **2**, 274-284

Dedov NV, Tran VH, Duke CC, Connor M, Christie MJ, Mandadi S, Roufogalis BD (2002) Gingerols: A novel class of vanilloid receptor (VR1) agonists. *British Journal of Pharmacology* **137 (6)**, 793-798

Dhanasekaran DN, Reddy EP (2008) JNK signaling in apoptosis. *Oncogene* **27 (48)**, 6245-6251

Dixon N, Wong LS, Geerlings TH, Micklefield J (2007) Cellular targets of natural products. *Natural Product Reports* **24**, 1288-1310

Duhovny D, Nussinov R, Wolfson HJ (2002) Efficient unbound docking of rigid molecules. In: Guigó R, Gusfield D (Eds) *Algorithms in Bioinformatics: Lecture Notes in Computer Science*, Springer Berlin, pp 185-200

Gidalevitz T, Biswas C, Ding H, Schneidman-Duhovny D, Wolfson HJ, Stevens F, Radford S, Argon Y (2004) Identification of the N-terminal peptide binding site of glucose regulated protein 94. *The Journal of Biological Chemistry* **279 (16)**, 16543-16552

Gordaliza M (2007) Natural products as leads to anticancer drugs. *Clinical and Translational Oncology* **9 (12)**, 767-776

Gore M, Bagchi P, Desai NS, Kar A (2010) Ayur-informatics: Establishing an *in-silico*-Ayurvedic medication for Alzheimer's disease. *International Jour-*

nal of Bioinformatics Research **2 (1)**, 33-37

Gu C, Zeng T, Li Y, Xu Z, Mo Z, Zheng C (2009) Structure-function analysis of mutant RNA-dependent RNA polymerase complexes with VPg. *Biochemistry (Moscow)* **74 (10)**, 1132-1141

Hanim S, Habib M, Makpol S, Hamid NAA, Das S, Ngah WZW, Yusof YAM (2008) Ginger extract (*Zingiber officinale*) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. *Clinics* **63 (6)**, 807-813

Inbar Y, Schneidman-Duhovny D, Halperin I, Oron A, Nussinov R, Wolfson HJ (2005) Approaching the CAPRI challenge with an efficient geometry-based docking. *Proteins* **60**, 217-223

Iyer AK, Tran KT, Borysenko CW, Cascio M, Camacho CJ, Blair HC, Bahar I, Wells A (2007) Tenascin cytotactin epidermal growth factor-like repeat binds epidermal growth factor receptor with low affinity. *Journal of Cellular Physiology* **211 (3)**, 748-758

Joza N, Susin SA, Daugas E, Stanford WL, Cho SK, Li CY, Sasaki T, Elia AJ, Cheng HY, Ravagnan L, Ferri KF, Zamzami N, Wakeham A, Hakem R, Yoshida H, Kong YY, Mak TW, Zúñiga-Pflücker JC, Kroemer G, Penninger JM (2001) Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature* **410**, 549-554

Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* **28 (1)**, 27-30

Kang BH, Plescia J, Song HY, Meli M, Colombo G, Beebe K, Scroggins B, Neckers L, Altieri DC (2009) Combinatorial drug design targeting multiple cancer signaling networks controlled by mitochondrial Hsp90. *The Journal of Clinical Investigation* **119 (3)**, 454-464

Kang BH, Tavecchio M, Goel HL, Hsieh CC, Garlick DS, Raskett CM, Lian JB, Stein GS, Languino LR, Altieri DC (2011) Targeted inhibition of mitochondrial Hsp90 suppresses localised and metastatic prostate cancer growth in a genetic mouse model of disease. *British Journal of Cancer* **104 (4)**, 629-634

Lambert C, Leonard N, De Bolle X, Depiereux E (2002) ESyPred3D: Prediction of proteins 3D structures. *Bioinformatics* **18 (9)**, 1250-1256

Leav I, Plescia J, Goel HL, Li J, Jiang Z, Cohen RJ, Languino LR, Altieri DC (2010) Cytoprotective mitochondrial chaperone TRAP-1 as a novel molecular target in localized and metastatic prostate cancer. *The American Journal of Pathology* **176 (1)**, 393-401

Lovell SC, Davis IW, Arendall WB III, de Bakker PIW, Word JM, Prisant MG, Richardson JS, Richardson DC (2003) Structure validation by Cα geometry: Φ, ψ and Cβ deviation. *Proteins: Structure, Function, and Bioinformatics* **50**, 437-450

Maheswari U (2011) Molecular docking study of tripeptides VPP and IPP inhibiting angiotensin I converting enzyme to alleviate high altitude pulmonary edema. *International Journal of Genetics and Molecular Biology* **3 (1)**, 1-6

McGee H (2004) *On Food and Cooking: The Science and Lore of the Kitchen* (2nd Edn), Scribner, New York, 884 pp

Mills S, Bone K (2000) *Principles and Practices of Phytotherapy: Modern Herbal Medicine*, Livingstone Churchill, Edinburgh, 643 pp

Papa S, Skulachev VP (1997) Reactive oxygen species, mitochondria, apoptosis and aging. *Molecular and Cellular Biochemistry* **174 (1)**, 305-319

Pruitt KD, Tatusova T, Maglott TR (2007) NCBI reference sequences (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Research* **35**, D61-D65

Rochfort S (2005) Metabolomics reviewed: A new "omics" platform technology for systems biology and implications for natural products research. *Journal of Natural Products* **68 (12)**, 1813-1820

Sayle RA, Milner-White EJ (1995) RASMOL: Biomolecular graphics for all. *Trends in Biochemical Sciences* **20 (9)**, 374-376

Schneidman-Duhovny D, Inbar Y, Polak V, Shatsky M, Halperin I, Benyamini H, Barzilai A, Dror O, Haspel N, Nussinov R, Wolfson HJ (2003) Taking geometry to its edge: Fast unbound rigid (and hinge-bent) docking. *Proteins* **52 (1)**, 107-112

Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock: Servers for rigid and symmetric docking. *Nucleic Acids Research* **33**, W363-367

Schroeter H, Boyd CS, Ahmed R, Spencer JP, Duncan RF, Rice-Evans C, Cadenas E (2003) c-Jun N-terminal kinase (JNK)-mediated modulation of brain mitochondria function: New target proteins for JNK signalling in mitochondrion-dependent apoptosis. *Biochemical Journal* **372 (Part 2)**, 359-69

Sharma A, Chaudhary PP, Sirohi SK, Saxena J (2011) Structure modeling and inhibitor prediction ofNADP oxidoreductase enzyme from *Methanobrevibacter smithii*. *Bioinformation* **6 (1)**, 15-19

Sharma A, Nigam A (2010) Structure modeling of novel DNA glycosylase enzyme from oral pathogen *Streptococcus sanguinis*. *Bioinformation* **5 (3)**, 136-140

Shukla Y, Singh M (2007) Cancer preventive properties of ginger: A brief review. *Food and Chemical Toxicology* **45**, 683-690

Thompson MA (2004) ArgusLab 4.0.1 Seattle, WA Planaria Software LLC

Wang C, Youle RJ (2009) The role of mitochondria in apoptosis. *Annual Review of Genetics* **43**, 95-118

Waszkowycz B, Clark DE, Gancia E (2011) Outstanding challenges in protein–ligand docking and structure-based virtual screening. *Wiley Interdisciplinary Reviews: Computational and Molecular Science* **1 (2)**, 229-259

Xia R, Dekermendjian K, Lullau E, Dekker N (2011) TRPV1: A therapy target that attracts the pharmaceutical interests. *Advances in Experimental Medicine and Biology* **704**, 637-665

Zhang C, Vasmatzis G, Cornette JL, DeLisi C (1997) Determination of atomic desolvation energies from the structures of crystallized proteins. *Journal of Molecular Biology* **267 (3)**, 707-26

Ziglioli F, Frattini A, Maestroni U, Dinale F, Ciuffreda M, Cortellini P (2009) Vanilloid-mediated apoptosis in prostate cancer cells through a TRPV-1 dependent and a TRPV-1-independent mechanism. *Acta Biomedica* **80**, 13-20